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ORIGINAL ARTICLE

Production of Matrix Metalloproteinases in Human Cultured Mast Cells: Involvement of Protein Kinase C–Mitogen Activated Protein Kinase Kinase–Extracellular Signal-regulated Kinase Pathway

Masahiro Kimata[1], Masayuki Ishizaki[1], Hiroyuki Tanaka[1], Hiroichi Nagai[1] and Naoki Inagaki[1]

ABSTRACT

Background: Matrix metalloproteinases (MMPs) have been reported to play crucial roles in the migration of inflammatory cells through basement membrane components. To confirm the role of mast cells as a source of MMPs, we investigated the production of MMP and its pathway in human cultured mast cells (HCMC). We also investigated the production of tissue inhibitors of metalloproteinase (TIMPs).

Methods: HCMC was stimulated with phorbor 12-miristate 13-acetate (PMA) and/or calcium ionophore A23187 (A23187), and the resulting MMP production was evaluated by gelatin zymography and western blotting. Expression of MMP and TIMP mRNA was also examined. Granulocyte macrophage-colony stimulating factor (GM-CSF) was measured by ELISA and activation of extracellular signal-regulated kinase (ERK) was evaluated by western blotting.

Results: We detected the *de novo* synthesis of MMP-9 in HCMC after stimulation with PMA and found that the synthesis was mediated through protein kinase C–mitogen activated protein kinase kinase (MEK)–ERK pathway. The MMP-9 production induced by PMA was suppressed by simultaneous treatment with A23187, whereas GM-CSF production was potentiated. We also detected the expression of mRNA for membrane-type 1 (MT1)-MMP, TIMP-1 and TIMP-2 after stimulation with PMA. Glucocorticoids and flavonoids inhibited MMP-9 production, and TIMPs and MMP inhibitors inhibited the gelatinolytic activity of mast cell-derived MMP-9. Furthermore, phenylmethylsulfonyl fluoride, a protease inhibitor, inhibited the conversion from proMMP-9 to active MMP-9.

Conclusions: These results suggest that the human mast cell is a leading member of MMP production, and the production, activation and activity are controllable by pharmacological agents.

KEY WORDS

human cultured mast cells, matrix metalloproteinase-9, membrane type matrix metalloproteinase, tissue inhibitor of metalloproteinase-1, tissue inhibitor of metalloproteinase-2

INTRODUCTION

Matrix metalloproteinases (MMP) are a family of zinc- and calcium-dependent endopeptidases capable of proteolytically degrading many of the components of the extracellular matrix.[1] MMP are produced by not only structural cells such as fibroblasts, endothelial cells and epithelial cells,[2] but also by inflammatory cells such as macrophages,[3] lymphocytes,[4] neutrophils[5] and eosinophils.[6,7] MMP are secreted as la-

[1]Department of Pharmacology, Gifu Pharmaceutical University, Gifu, Japan.
Correspondence: Naoki Inagaki Ph.D., Department of Pharmacology, Gifu Pharmaceutical University, 5–6–1 Mitahorahigashi, Gifu 502–8585, Japan.
Email: inagakin@gifu-pu.ac.jp
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tent forms followed by proteolytic processing to active forms, and involved in physiologic processes such as development, angiogenesis and wound healing, and in pathologic conditions such as tumor invasion and inflammation.[2,8] The proteolytic activation of latent forms and enzymatic activities of active forms of MMP are inhibited by endogeneous inhibitors, tissue inhibitors of metalloproteinase (TIMP) that form a 1:1 complex with MMP.[9] The balance between the levels of MMP and TIMP is thought to be a critical factor in regulating the breakdown of connective tissues by MMP, which is the case in pulmonary emphysema.[10] While the majority of the MMP are secreted as soluble enzymes into the extracellular milieu, a subset of MMP has been identified in recent years to contain additional sequences downstream of the hemopexin-like domain capable of anchoring on plasma membrane.[11,12] Named after the putative transmembrane domains as membrane-type matrix metalloproteinase 1 to 5 (MT1-, MT2-, MT3-, MT4- and MT5-MMPs), these enzymes have been proposed to be the master switches of extracellular matrix turnover based on the purported ability of MT-MMP to activate other MMP.[13,14]

MMP has been recognized because of its participation in allergic inflammatory diseases such as bronchial asthma, especially in remodeling and fibrosis.[15-17] These enzymes make it possible for inflammatory cells to infiltrate inflammatory sites. Especially, eosinophil transmigration is well explained with regard to the role of MMP-9.[18] The production of MMP from inflammatory cells such as eosinophils,[6,7] neutrophils[5] and macrophages[3] has been well investigated *in vivo* and *in vitro*, although the production of MMP from mast cells is less understood. As for mast cells, several investigators have reported the production of MMP from some types of mast cells (mouse mast cells, dog mastocytoma cells and human mast cells).[19-22] However, the production pathway of MMP from mast cells and its regulation have not been investigated yet. In this report, therefore, we investigated the MMP production and its pathway using human cultured mast cells (HCMC).

METHODS

AGENTS

Actinomycin D (Act.D), cycloheximide (CHX), aminophenylmercuric acetate (APMA), phenylmethylsulfonyl fluoride (PMSF), and staurosporine were purchased from Sigma (St. Louis, MO, USA). U0126 and PD98059[23] were obtained from Promega (Madison, WI, USA) and Calbiochem (La Jolla, CA, USA), respectively. Bisindolylmaleimide II (BIS II; Alexis Co., San Diego, CA, USA), prednisolone phosphate (Banyu Co., Tokyo, Japan), dexamethasone phosphate (Takeda Chemical Industries, LTD., Osaka, Japan), luteolin (Sigma), baicalein (Extrasynthese, Genay, France), quercetin (Extrasynthese), TIMP-1 and TIMP-2 (Fuji Chemical Industries, LTD, Toyama, Japan) were obtained from each respective company. AG-3340,[24] marimastat,[24] CGS27023A[25] and Ro32-3555[26] were synthesized. Concentrations of the agents employed were decided according to our preliminary experiments.

HUMAN MAST CELL CULTURE

HCMC were obtained by culturing human umbilical cord blood cells using a method described previously.[27] Briefly, heparinized umbilical cord blood was donated from mothers with informed consent and mononuclear cells (MNC) were separated from the blood by lymphocyte separation medium (ICN Biomedicals Inc., Aurora, OH, USA) gradient. $CD34^+$ cells were purified from the MNC fraction by using a $CD34^+$ progenitor cell isolation kit (Miltenyi Biotec, Bergisch Gladbach, Germany) and magnetic cell sorting system (MACS; Miltenyi Biotec). Purified $CD34^+$ cells were cultured in an α-minimum essential medium (GIBCO-RBL Laboratories, Gland Island, NY, USA) containing 15% fetal bovine serum (Filtron Pty Ltd., Brooklyn, Australia) in the presence of 80 ng/ml human recombinant stem cell factor (SCF; Kirin Brewery, Maebashi, Japan) and 50 ng/ml human recombinant interleukin-6 (Kirin). Cells were harvested weekly and resuspended in a fresh media. The purity of mast cells was determined by toluidine blue and May-Gruenwald and Giemsa staining. Immunoperoxidase staining for tryptase and chymase was performed by the method previously established.[28] HCMC employed in the present study consisted of a more than 15 week-old culture with >99% purity. The HCMC were almost 100% positive for tryptase and 14% positive for chymase.

DETECTION OF EXTRACELLULAR-REGULATED KINASE (ERK) AND MMP-9 BY WESTERN BLOTTING

HCMC were stimulated with phorbor 12-myristate 13-acetate (PMA) or anti-IgE for indicated periods as reported previously.[29] To stimulate HCMC IgE-dependently, HCMC were incubated in the presence of 1 μg/ml human myeloma IgE (Chemicon Int. Inc., Temecula, CA, USA) at 37℃ overnight and then challenged with 4 μg/ml anti-human IgE (DAKO, Clostrup, Denmark). For the detection of ERK, stimulated cells (1×10^5 cells/lane) were lysed with ice-cold lysis buffer (50 mM Tris-HCl, pH 7.5; 0.15 M NaCl, 1% IGEPAL CA-630, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 1 mM EDTA and 1mM Na_3VO_4). For the detection of MMP-9, HCMC (5×10^5 cells/ml) were stimulated for indicated periods, and then the supernatants were separated. Analysis of samples by SDS-PAGE and transfer to PVDF membrane (PVDF-Plus, pore size=0.45 micron; Micron Separations, Inc., Westboro, MA, USA) were performed as described

elsewhere.[30] ERK and MMP-9 were detected with the antibodies against phosphorylated forms of ERK (Phospho-p44/42 MAP Kinase antibody; New England Biolabs, Inc., Bevery, MO, USA) and MMP-9 (Fuji Chemical Ind., LTD), respectively. The anti-MMP-9 antibodies detected both proMMP-9 and active MMP-9. Horseradish peroxidase-conjugated antibodies against rabbit IgG (for ERK; Promega, Madison, WI, USA) and mouse IgG (for MMP-9; Promega) were used as secondary antibodies. Finally, the proteins were visualized by the ECL-plus system (Amersham).

DETECTION OF MMP PRODUCTION BY GELATIN ZYMOGRAPHY

HCMC at a concentration of 5×10^5 cells/ml were incubated with PMA in serum-free medium for indicated periods and culture supernatants were harvested. Gelatin zymography was performed as described previously with some modifications.[31] The culture supernatant was electrophoresed in 10% polyacrylamide gels containing 1 mg/ml gelatin in the presence of SDS under non-reducing conditions. Gels were then washed three times in 50 mM Tris-HCl, pH 7.5, containing 2.5% Triton X-100, 10 mM $CaCl_2$ and 1 mM $ZnCl_2$, and further incubated in Triton X-100 buffer at 37℃ for 24 hr. Following incubation, the gels were stained with Coomassie brilliant blue R 250. Destaining visualized clear zones of lysis against a blue background indicated gelatinase activity. When the effects of drugs on MMP production were examined, drugs were added to HCMC suspension 10 min before PMA stimulation.

EFFECTS OF TIMPS AND MMP INHIBITORS ON MMP-9 ACTIVITY

Effects of TIMPs and MMP inhibitors on MMP-9 activity were examined by gelatin zymography. HCMC were incubated with PMA at a concentration of 10 nM for 48 hr, and the supernatant was separated and used as a source of MMP-9. The supernatant was electrophoresed in gelatin containing gels, and the gels were further incubated for 24 hr in the presence of TIMPs and MMP inhibitors. EDTA, which chelates calcium and zinc ions, was tested as a positive control.

GM-CSF PRODUCTION

HCMC were resuspended at 5×10^5 cells/ml in fresh culture media and challenged with PMA and calcium ionophore A23187 (A23187; Sigma) for 6 hr. GM-CSF in the supernatant was quantified using a commercial ELISA kit (R&D Systems, Inc., Minneapolis, MN, USA).

ISOLATION OF RNA AND REVERSE TRANSCRIPTASE-POLYMERASE CHAIN REACTION (RT-PCR)

Total cellular RNA was isolated from stimulated HCMC using Isogen (Nippon gene Co, Tokyo, Japan). The first-strand cDNA was synthesized from 1 μg of total RNA using the superscript preamplification system (GIBCO-RBL Laboratories) according to the manufacture's instruction. PCR was performed as follows: 94℃, 90 seconds of denaturing; 62℃, 90 seconds of annealing; and 72℃, 90 seconds of extension, for 30 cycles (β-actin) or 32 cycles (others). Primers used were as follows:β-actin, sens: 5'-CAAGAGATGG CCACGGCTGCT-3', antisense: 5'-TCCTTCTGCATC CTGTCGGCA-3'; MMP-9, sens: 5'-GTGCTGGGCTG CTGCTTTGCTG-3', antisense: 5'-GTCGCCCTCAAA GGTTTGGAAT-3'; MT1-MMP, sens: 5'-TCGGCCCA AAGCAGCAGCTTC-3', antisense: 5'-CTTCATGGTG TCTGCATCAGC-3'; TIMP-1, sens: 5'-TGCACCTGTG TCCCACCCCACCCACAGACG-3', antisense: 5'-GGC TATCTGGGACCGCAGGGACTGCCAGGT-3'; TIMP-2, sens: 5'-CAGCCGAGCAGCCACATCG-3', antisens e: 5'-TGAGGCTGTTGTCATACTTCTC-3'.PCR products were analyzed by agarose gel electrophoresis.

RESULTS

MMP PRODUCTION FROM HCMC

HCMC were stimulated with 10 nM PMA for 12–96 hours and gelatinolytic activity in the supernatant was examined by gelatin zymography. As shown in Figure 1A, 3 gelatinolytic bands were detected. According to the position, upper bands and middle bands were considered to be proMMP-9 and active MMP-9, respectively. The band for proMMP-9 was observed from 12 hours after the stimulation and increased up to 96 hours, and that for active MMP-9 was detected from 24 hours after the stimulation. The lower dim bands only detected after 96 hours seemed to be MMP-2 according to the report by Kanbe *et al.*[19]

Because it was considered that gelatin zymography might be less quantitative, we carried out western blot analysis to confirm the MMP-9 production. We employed monoclonal antibodies recognizing both proMMP-9 and active MMP-9. As shown in Figure 1B, similar results were obtained in western blot analysis, although slightly longer periods were needed for detection than in the case of gelatin zymography. These results supported the reliability of gelatin zymography, and therefore we employed it in the following experiments.

Next, we attempted to characterize the production of MMP-9 production HCMC. Initially, we examined the effect of APMA, an MMP activator, on active MMP-9 production. After 48 hours of incubation with PMA at a concentration of 10 nM, the supernatant was collected and further incubated with 1 mM

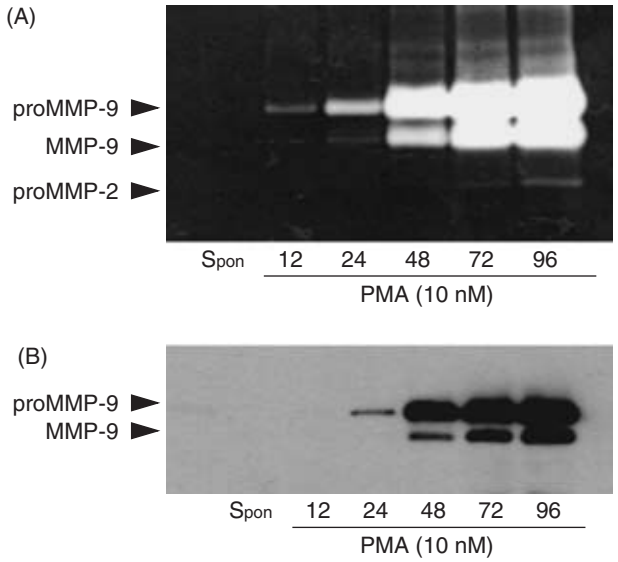

Fig. 1 MMP production from HCMC. HCMC were suspended in serum-free medium and incubated with 10 nM PMA for indicated periods. ProMMP-9 bands, active MMP-9 bands and proMMP-2 bands were detected in gelatin zymography analysis (**A**). Western blot analysis were performed with PMA stimulated HCMC supernatants by using anti-MMP-9 antibodies (**B**). Horseradish peroxidase-conjugated antibody was used as a secondary antibody. Proteins were visualized using the ECL-plus system. Each result is a representative of three independent experiments.

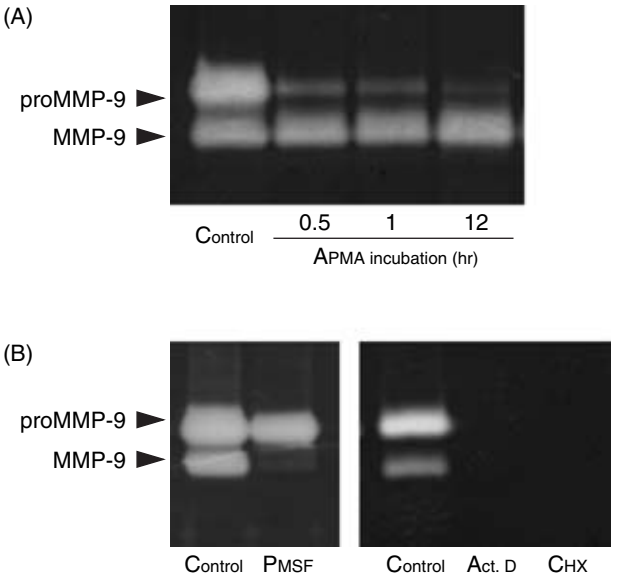

Fig. 2 Effects of agents on the activation and production of MMP-9. After incubation of HCMC with PMA (10 nM) for 48 hours, the supernatant was collected and further incubated with APMA (1 mM) for indicated periods (**A**). PMSF (1 mM), Act.D (1 μg/ml) and CHX (1 μg/ml) were added to HCMC 10 minutes before PMA (10 nM) stimulation, and then incubated for 48 hours (**B**). The samples were subjected to gelatin zymography under nonreducing conditions. Each result is a representative of three different experiments.

APMA for 0.5−12 hours. As shown in Figure 2A, proMMP-9 band diminished gradually dependent on the period of incubation and only active MMP-9 band was detected after 12 hours of incubation. We next examined the effects of PMSF, Act.D and CHX on MMP-9 production from HCMC. As shown in Figure 2B, when HCMC was treated with 1 mM PMSF, active MMP-9 band was not detectable. Some serine proteinases from mast cells might contribute to the conversion of MMP-9 (92 KDa band to 82 KDa band) in the culture media, which can be inhibited by chemical serine proteinase inhibitor, PMSF. Furthermore, both 1 μg/ml Act.D and 1 μg/ml CHX abolished MMP-9 bands completely. These results indicate that MMP-9 is synthesized *de novo* upon stimulation with PMA.

CHARACTERIZATION OF MMP-9 PRODUCTION PATHWAY

To determine the MMP-9 production pathway, we examined the effects of protein kinase C (PKC) inhibitors, staurosporine and Bis II, and mitogen-activated protein kinase kinase (MEK) inhibitors, U0126 and PD98059. The specificity of Bis II has been confirmed by Miura *et al.*,[32] and that of U0126 was established previously by several investigators.[23] As shown in Figure 3, MMP-9 production was completely inhibited by both the PKC inhibitors and MEK inhibitors. Accordingly, MMP-9 was produced through the PKC−MEK−ERK pathway.

When we stimulated HCMC through FcεRI, MMP-9 production was not induced to any extent (data not shown). In order to investigate the difference between PMA-induced stimulation and FcεRI-mediated stimulation, we examined the ERK activation through these stimulation methods. As shown in Figure 4, PMA-induced activation of ERK was stronger and longer lasting compared to FcεRI-mediated activation. This difference, therefore, may be critical regardless of whether MMP-9 was produced or not.

We compared the production of MMP-9 and GM-CSF from HCMC simultaneously stimulated with PMA and A23187. As shown in Figure 5A, simultaneous treatment with A23187 profoundly inhibited the PMA-induced MMP-9 production. In contrast, when HCMC were stimulated with PMA and A23187, dramatically increased amounts of GM-CSF were produced (Fig. 5B).

EFFECTS OF GLUCOCORTICOIDS AND FLAVONOIDS ON MMP-9 PRODUCTION FROM HCMC

We attempted to identify efficient agents that could

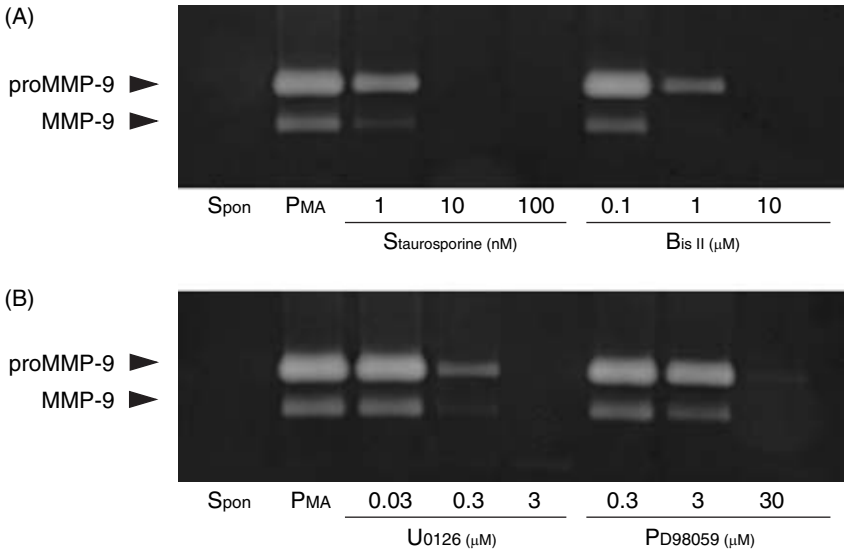

Fig. 3 Effects of PKC inhibitors (**A**) and MEK inhibitors (**B**) on MMP-9 production from HCMC. Staurosporine (1–100 nM), Bis II (0.1–10 μM), U0126 (0.03–3 μM) and PD98059 (0.3–30 μM) were added to HCMC 10 minutes before PMA (10 nM) stimulation, and then incubated for 48 hours. The samples were then subjected to gelatin zymography under nonreducing conditions. Each result is a representative of three different experiments.

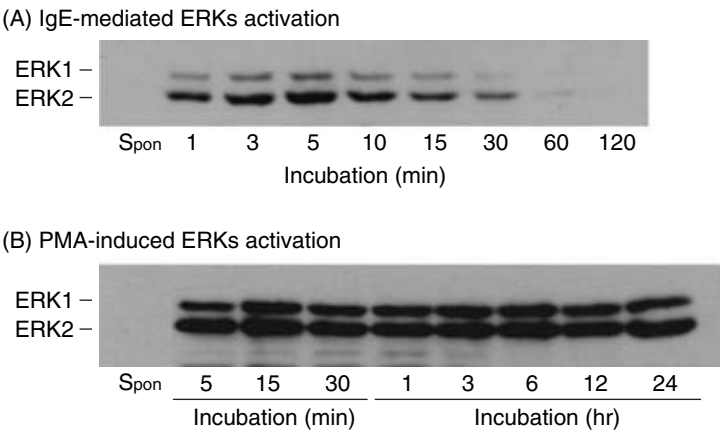

Fig. 4 IgE-mediated (**A**) and PMA-induced (**B**) ERK activation in HCMC. Sensitized HCMC were challenged with anti-IgE (4 μg/ml) or non-sensitized HCMC were stimulated with PMA (10 nM) for the indicated periods. The total cellular proteins were analyzed by SDS-PAGE and western blotting. Phosphorylation of ERK was detected with phospho-specific antibody. Horseradish peroxidase-conjugated antibody was used as a secondary antibody. Proteins were visualized using the ECL-plus system. Each result is a representative of three different experiments. Spon: spontaneous.

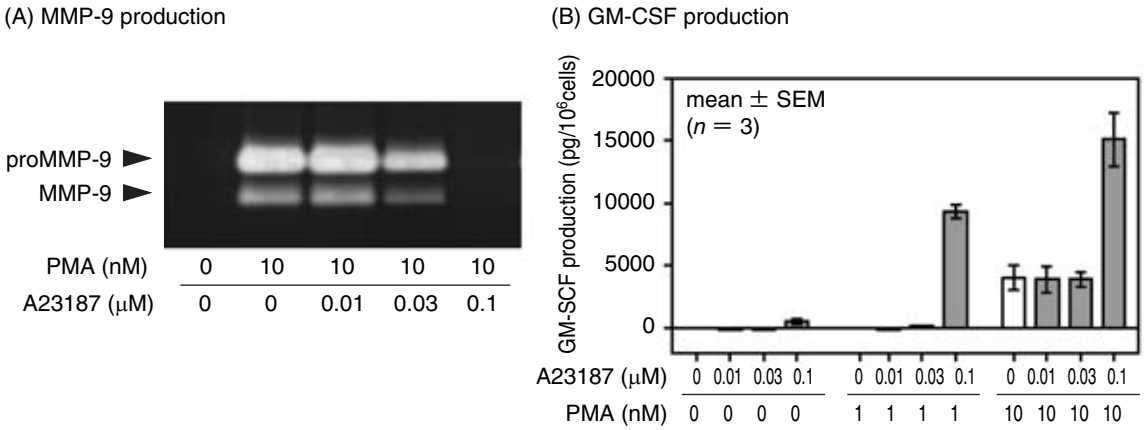

Fig. 5 Combined effect of A23187 and PMA on MMP-9 (**A**) and GM-CSF (**B**) production from HCMC. HCMC were stimulated simultaneously with the indicated concentrations of PMA and A23187. For the evaluation of MMP-9, cells were incubated for 48 hours and then gelatin zymography was performed. For the GM-CSF production, cells were incubated for 6 hours and GM-CSF in the supernatant was quantified by ELISA. Result in (**A**) is a representative of three different experiments and values in (**B**) are the mean ± SEM of three different experiments.

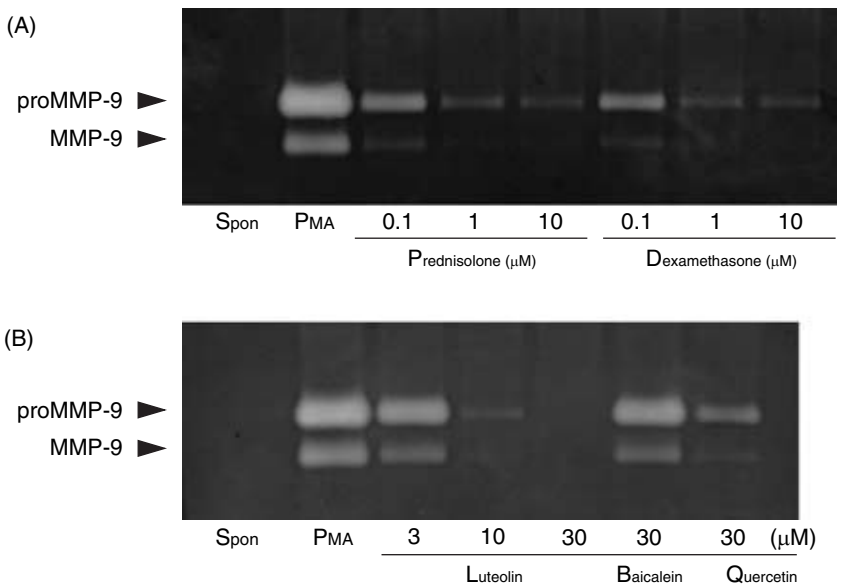

Fig. 6 Effects of glucocorticoids (**A**) and flavonoids (**B**) on MMP-9 production from HCMC. Prednisolone (0.1–10 μM), dexamethasone (0.1–10 μM), luteolin (3–30 μM), baicalein and quercetin (30 μM) were added to HCMC before PMA (10 nM) stimulation, and then incubated for 48 hours. The samples were then subjected to gelatin zymography under nonreducing conditions. Each result is a representative of three different experiments.

regulate MMP-9 production from human mast cells. As shown in Figure 6A, prednisolone and dexamethasone inhibited MMP-9 production in a dose-dependent manner. Recently, we reported that certain flavonoids powerfully inhibited FcεRI-mediated mediator releases in HCMC.[33] As shown in Figure 6 B, the flavonoids, luteolin and quercetin, inhibited the PMA-induced MMP-9 production.

EFFECTS OF MMP INHIBITORS ON MMP-9 ACTIVITY

Recently, many MMP inhibitors including endogeneous MMP inhibitors, TIMP-1 and TIMP-2, have been reported.[9] Therefore, we investigated the effects of these MMP inhibitors on HCMC-derived gelatinolytic activity. As shown in Figure 7A, TIMP-1 and TIMP-2 clearly inhibited the gelatinolytic activity.

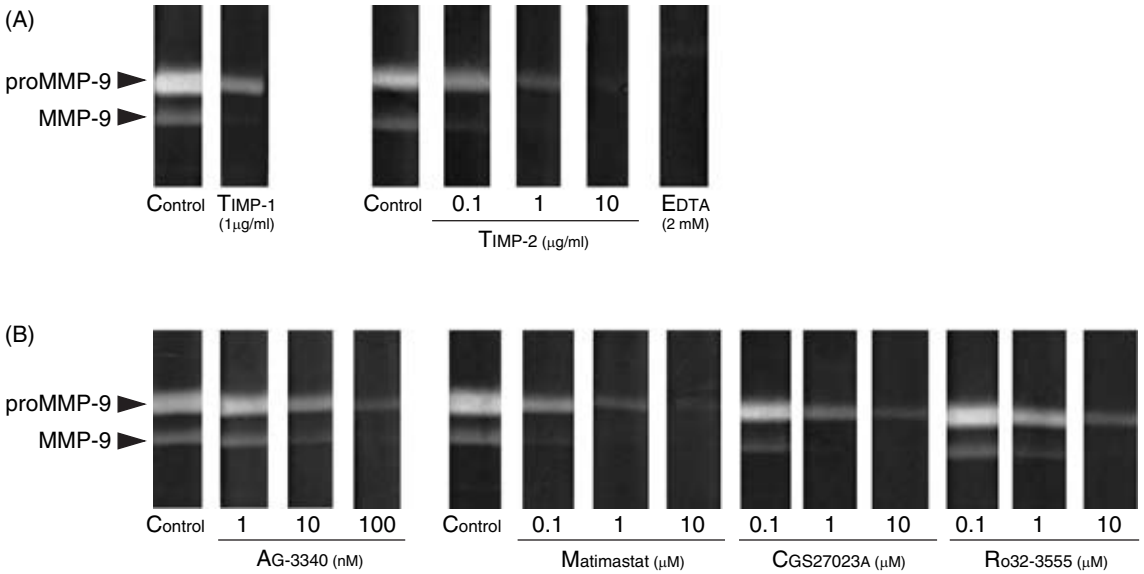

Fig. 7 Effects of TIMPs and MMP inhibitors on MMP-9 activity . HCMC were incubated with PMA (10 nM) for 48 hours and the supernatant was collected. This supernatant was electrophoresed with gelatin containing gels. The gels were incubated for 24 hours with TIMPs (**A**) or MMP inhibitors (**B**). TIMP-1, TIMP-2, AG-3340, marimastat, CGS27023A, and Ro32-3555 were used in indicated concentrations. EDTA, which chelates calcium and zinc ions was tested as a positive control. Each result is a representative of three different experiments.

Synthesized MMP inhibitors such as AG-3340, marimastat, CGS27023A and Ro32–3555 also inhibited the gelatinolytic activity (Fig. 7B). EDTA abolished the gelatinolytic activity.

PMA-INDUCED MMP-9, MT1-MMP, TIMP-1 AND TIMP-2 mRNA EXPRESSION IN HCMC

To determine whether MMPs and TIMPs in addition to MMP-9 and MMP-2 are produced or not, we investigated the mRNA expression 6–96 hours after PMA stimulation. As shown in Figure 8, the expression of mRNA for MMP-9 and MT1-MMP was detectable from 6 hours after stimulation and increased up to 96 hours. On the other hand, TIMP-1 and TIMP-2 mRNAs were detectable even in non-stimulated cells and the expression was gradually increased by PMA stimulation during the incubation.

DISCUSSION

In this study using human mast cells, we demonstrated for the first time that MMP-9 is produced through the PKC–MEK–ERK pathway, and that this production can be regulated by several agents, such as glucocorticoids and flavonoids. Furthermore, we found that not only MMP-9 but also MT1-MMP, TIMP-1 and TIMP-2 mRNAs were expressed after PMA stimulation.

Recently, the functions of MMP have been recognized, because they are involved in physiologic processes such as development, angiogenesis and wound healing, and in pathologic conditions such as tumor invasion and inflammation.[2,8] MMP is also reported to play a role in the remodeling of airways and fibrosis.[15-17] As for inflammatory processes, MMP-9 and MMP-2 have been especially well investigated, because these enzymes are potentially involved in the processes of cellular infiltration from circulation to inflammatory sites through the endothelial and epithelial basement membranes.[34] Results from *in vitro* studies suggest that lymphocytes,[4] neutrophils[5] and eosinophils[6,7] pass through the basement membrane by degrading components with their own MMP-9 and MMP-2. Although there are many reports that suggest that MMP production originates from these inflammatory cells, there is little investigation of mast cells. In contrast to other inflammatory cells, mast cells are located in inflammatory sites, and after stimulation these cells produce a variety of cytokines (such as TNF-α, MIP-1α and IL-8),[35-38] which induce the migration of inflammatory cells. Therefore, mast cell-derived MMPs could degrade extracellular matrix around mast cells to allow other inflammatory cells easier migration and accumulation.

Fang *et al.* reported that SCF-induced MMP-9 production in dog BR mastocytoma cells.[21] Furthermore, Tanaka *et al.* reported that MMP-9 production is inhibited by SCF-induced activation in mouse bone marrow-derived mast cells.[20] In contrast in HCMC, MMP-9 production is neither induced nor inhibited by SCF (data not shown). The difference may contribute to the characteristic of these types of mast cells. Therefore, in order to investigate the roles of

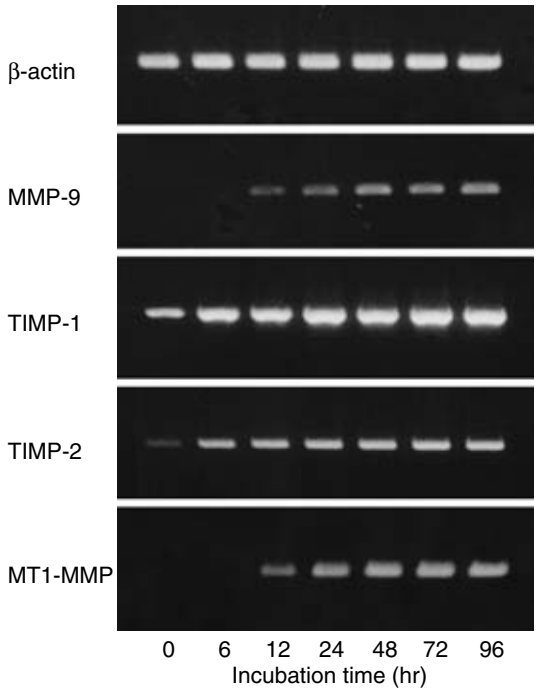

Fig. 8 MMP mRNA expression in HCMC stimulated with PMA. HCMC were stimulated with PMA (10 nM) for 6–96 hours, and analyzed mRNA expression for MMP-9, MT1-MMP, TIMP-1 and TIMP-2 by RT-PCR. The results presented are representative of three different experiments.

mast cells in human pathologic conditions, human mast cells should be employed. Recently, the production of MMP-9 from human mast cells has been reported in HMC-1, HCMC and tissue mast cells (lung, skin and synovial membrane).[19] In this report, we further investigated the MMP-9 production pathway from HCMC and its regulation in detail.

At first, we confirmed 92 KDa and 82 KDa bands are associated with MMP-9 by using APMA and PMSF. An RNA synthesis inhibitor, Act.D, and a protein synthesis inhibitor, CHX, completely inhibited the MMP-9 production. The PMA-induced MMP-9 production, therefore, is due to *de novo* synthesis. Actually, gelatinolytic activity was not detected at all in the non-stimulated HCMC supernatant. We next confirmed that MMP-9 production is completely inhibited by both PKC inhibitors and MEK inhibitors. Therefore, it can be concluded that this MMP-9 production is induced by the PKC–MEK–ERK pathway. Furthermore, PMA-induced MMP-9 production was inhibited by A23187 in a dose-dependent manner. On the other hand, GM-CSF production was induced by PMA and A23187 synergistically. Therefore, this MMP-9 production is regulated in a different way from cytokine production such as GM-CSF. Furthermore, although A23187 induce GM-CSF production from HCMC, it is unable induce MMP-9 production (data not shown). The mechanism of calcium signaling which leads to two separate phenomena (down-regulation of MMP-9 and up-regulation of GM-CSF) should be investigated furthermore. We previously reported that IgE-mediated mediator release such as leukotrienes from HCMC are induced through both the PKC and Ca^{2+} pathways.[29] Therefore, MMP-9 production might not be induced in IgE-mediated stimulation due to the increase of intracellular Ca^{2+} levels. Another possibility for the explanation of why IgE-mediated stimulation could not induce MMP-9 production is the difference of ERK pathway activation. In PMA-stimulated HCMC, ERK activation is detectable even at 24 hours after stimulation, whereas IgE-mediated ERK activation is not detectable after 60 minutes. Thus, prolonged activation of the ERK may play a role in MMP-9 production.

The role of MMP derived from eosinophils and neutrophils is somewhat obvious. These cells seem to use MMPs to degrade extracellular matrix, and then migrate to the inflammatory site.[5-7] In contrast, mast cells are settled in tissues and may not always need to migrate. Here, we suggest two possibilities that explain why mast cells produce MMPs. It is already reported that a large number of mast cells are observed in chronic inflammatory sites.[39] Therefore, mast cell MMPs may play some role in the accumulation of mast cell in chronic inflammatory sites. As already described, the other possibility is that mast cell-derived MMPs destruct matrix around the mast cells in order to facilitate the migration of other inflammatory cells. Based on these viewpoints, the inhibition of MMPs production from mast cells might be one of the strategies involved in the inhibition of chronic inflammation such as asthma and chronic obstructive pulmonary disease.[40]

Possible strategies for inhibiting the degradation of extracellular matrix by mast cells are as follows: 1) to inhibit the MMP production from mast cells, 2) to inhibit the gelatinolytic activity of mast cell-derived MMPs, and 3) to inhibit the conversion to active MMP-9 because proMMP-9 can not degrade extracellular matrix. Glucocorticoids, luteolin and quercetin inhibited MMP-9 production from HCMC. These agents are known to be inhibitors of IgE-mediated mediator release from mast cells,[33,41] and these agents are also confirmed as inhibitors of MMP-9 production. Furthermore, we confirmed that MMP-9 activity is inhibited by both endogeneous MMP-9 inhibitors and synthesized MMP-9 inhibitors. These results indicate that mast cell-induced gelatinolytic activity is controllable by some agents. As for protease inhibitors such as PMSF, which inhibit the conversion to active MMP-9, there is retention of MMP-9 as a latent form.

The balance between MMPs and TIMPs is important for the balance of tissue destruction and reconstruction.[10] Although we first detected MMP-9 and

MMP-2 by gelatin zymography, we further investigated whether other MMPs and TIMPs are produced from PMA-stimulated HCMC or by RT-PCR. In this study, we detected TIMP-1, TIMP-2 and MT1-MMP mRNA expression. TIMP-1 and TIMP-2 mRNAs were detected also in unstimulated cells, whereas MMP-9 mRNA was not detected under the same conditions. Therefore, constitutive expression of TIMPs in tissue resident mast cells may be important for homeostasis. Because mast cells contain the proteolytic enzymes tryptase and chymase, it follows that these enzymes easily activate proMMP-9 to active form.[21] Accordingly, the expression of TIMPs may regulate the activation of endogeneous proMMP-9 by the mast cell enzymes. From this data, non-activated mast cells might participate in extracellular matrix turnover in the process of physiological tissue remodeling especially for tissue reconstruction. Additionally, the mRNA expression of MT1-MMP is noticeable, because this membrane type enzyme activates proMMP-2 more potently than active MMP-3.[24,25] We failed to detect MMP-3 production by HCMC in western blotting analysis (data not shown), although the detection of MT1-MMP revealed the possibility that mast cells can activate mast cell-derived MMP-2 by their surface-expressed MT1-MMP. This must be a reasonable scenario for the activation of mast cell-derived MMP-2.

In summary, this report presents the MMP-9 production pathway in human mast cells and the regulation of MMP-9 production. Importantly, human mast cells produce not only MMP-9, MMP-2 and MT1-MMP but also TIMP-1 and TIMP-2. The balance between MMPs and TIMPs derived from mast cells may control the pathogenesis of chronic inflammation.

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